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脓毒症患儿内生吗啡动态变化及临床意义*

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摘要 目的:检测脓毒症患儿血清内生吗啡(EM)动态变化,探讨脓毒症患儿血清 EM 水平的临床意义。**方法:**选择 2017 年 6 月-2018 年 2 月华中科技大学同济医学院附属同济医院收治的 30 例伴有严重脓毒症或脓毒症休克的患儿作为脓毒症组,30 例全身炎症反应综合征(SIRS)患儿作为 SIRS 组,30 例健康儿童作为对照组。脓毒症组、SIRS 组及对照组分别于入组后第 1、3、6、9 天抽取外周静脉血,采用免疫荧光法检测血清降钙素原(PCT)水平,采用酶联免疫吸附试验(ELISA)测定血清 EM 水平,采用流式细胞术(FCM)检测细胞免疫功能。**结果:**SIRS 组、脓毒症组患儿的第 1、3、6 天血清 PCT 水平显著高于对照组,且随时间延长 PCT 水平逐渐降低,至第 9 天降至正常水平($P<0.05$),而脓毒症组与 SIRS 组间血清 PCT 水平无统计学差异($P>0.05$)。脓毒症组第 1、3、6 天血清 EM 水平均高于 SIRS 组($P<0.05$),第 9 天两组血清 EM 水平比较差异无统计学意义($P>0.05$)。脓毒症组 EM 水平随时间延长而降低,至第 9 天降至 SIRS 组的水平($P<0.05$)。与 SIRS 组相比,第 1 天脓毒症组的 CD3⁺T 细胞数量增多($P<0.05$),两组 CD4⁺、CD8⁺T 细胞数量、CD4⁺/CD8⁺ 比例比较无统计学差异($P>0.05$)。**结论:**脓毒症患儿中血清 EM 水平较高,有可能作为诊断脓毒症特异性较高的生物标志物。

关键词:脓毒症;内生吗啡;全身炎症反应综合征;生物标志物;免疫功能

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The Dynamic Change and Clinical Significance of Serum Endogenous Morphine in Children with Sepsis*

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ABSTRACT Objective: To detect the dynamic changes of plasma endogenous morphine (EM) in children with sepsis and to explore the clinical significance of serum EM level in children with sepsis. **Methods:** 30 children with severe sepsis or septic shock treated in our hospital from June 2017 to February 2018 were selected as sepsis group. 30 children with systemic inflammatory response syndrome (SIRS) as SIRS group and 30 healthy children as control group. Peripheral venous blood was collected from sepsis group, SIRS group and control group at 1 day, 3 days, 6 days, 9 days after admission respectively. Serum calcitonin (PCT) level was detected by immunofluorescence, serum EM was measured by enzyme linked immunosorbent assay (ELISA), and cell immunologic function was detected by flow cytometry (FCM). **Results:** The level of serum PCT in SIRS group and sepsis group was significantly higher than that of control group at 1 day, 3 days, 6 days, as time went on, the level of PCT decreased gradually, and decreased to normal level at 9 days ($P<0.05$). There was no significant difference in serum PCT level between sepsis group and SIRS group ($P>0.05$). The serum EM level in sepsis group was higher than that in SIRS group at 1 day, 3 days, 6 days ($P<0.05$). There was no significant difference in serum EM level between the two groups at 9 days ($P>0.05$). The level of EM in sepsis group decreased with time and decreased to SIRS level at 9 days ($P<0.05$). Compared with the SIRS group, the number of CD3⁺T cells in the sepsis group increased at 1 day ($P<0.05$), and there was no significant difference in the number of CD4⁺ and CD8⁺T cells and the proportion of CD4⁺/CD8⁺ in the two groups ($P>0.05$). **Conclusion:** Serum EM level in children with sepsis are relatively higher and may be used as biomarkers for the diagnosis of sepsis.

Key words: Sepsis; Endogenous morphine; Systemic inflammatory response syndrome; Biomarker; Immune function

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前言

脓毒症是由各种病原微生物引起的全身性炎症反应综合

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征,其发展迅速,若得不到及时有效的治疗,可导致脓毒性休克、多器官功能障碍等严重并发症,对患者生命造成极大的威胁^[12]。早期诊断及评估患者严重程度可使患者得到针对性的治疗,从而改善脓毒症患者的预后^[3-6]。近年来,相关研究报道降钙素原(procalcitonin, PCT)能够作为早期诊断和评估脓毒症严重程度的生物标志物,但其特异性和敏感性较低,并且 PCT 很难鉴别脓毒症和非感染引起的全身炎症反应综合征(systemic inflammatory response syndrome, SIRS)^[6-8]。有研究发现,严重脓毒症或脓毒症休克患者血清中内生吗啡(endogenous morphine, EM)的水平显著高于 SIRS 患者和普通脓症患者,脓症患者血清 EM 是中性粒细胞受炎症因子刺激后释放的,与机体免疫调节有关^[9]。因此 EM 有可能作为一个比 PCT 更好的脓毒症的新生物标志物。本研究通过观察脓毒症患儿血清 EM 的动态变化,旨在为脓毒症的早期诊断、预后判断寻求更好的生物活性指标。

1 对象与方法

1.1 研究对象

选择 2017 年 6 月-2018 年 2 月华中科技大学同济医学院附属同济医院收治的 30 例伴有严重脓毒症或脓毒症休克的患儿作为脓毒症组,年龄 1 月-13 岁,平均(5.21 ± 3.22)岁,男 17 例,女 13 例,所有患者均有明确感染灶,并符合脓毒症诊断标准^[10](至少满足以下 2 项:体温 $>38^{\circ}\text{C}$ 或 $<36^{\circ}\text{C}$,心率 >90 次/min,呼吸 >20 次/min,白细胞计数 $>12 \times 10^9/\text{L}$ 或 $<4 \times 10^9/\text{L}$); 30 例健康儿童作为对照组,年龄 9 月-12 岁,平均(5.78 ± 3.09)岁,男 16 例,女 14 例;30 例 SIRS 患儿作为 SIRS 组,年龄 4 月-13 岁,平均(5.35 ± 2.27)岁,男 15 例,女 15 例,各组间性别、年龄比较无统计学差异($P>0.05$)。脓毒症组、SIRS 组均需排除以下情况:近期接受手术治疗者,患有肿瘤或者血液病者,近期使用炎症因子药物者。且所有研究对象的家长均签署知情同意书,

经华中科技大学同济医学院附属同济医院伦理委员会批准。

1.2 标本采集与检测

所有研究对象在入组后第 1、3、6、9 天,于清晨空腹采取外周静脉血 2 mL,在 4°C 离心 10 min, 1500 r/min,收集上层血清,并保存于 -80°C 冰箱。PCT 检测试剂盒(免疫荧光层析法,武汉明德生物科技股份有限公司)、免疫定量分析仪(HR201,深圳华科瑞科技有限公司)检测血清 PCT 水平。吗啡特异性酶联免疫吸附试验(enzyme-linked immunosorbent assay, ELISA)试剂盒(EHC9075,欣博盛生物科技股份有限公司)、全自动酶标仪(ELx800,美国 Bio Tek 公司)检测血清中 EM 水平。流式细胞术(flow cytometry, FCM)检测 SIRS 组、脓毒症组的细胞免疫功能。于入组后第 1 天抽取抗凝血 100 μL ,加入兔抗人 CD4-FITC/CD8-PE/CD3-PerCP(美国 BD 公司),避光,室温 30 min, PBS 洗涤后,采用流式细胞仪(FACSCalibur/Calibur,美国 BD 公司)检测 CD3⁺、CD4⁺、CD8⁺T 细胞数量,计算 CD4⁺/CD8⁺ 比例。

1.3 统计学方法

采用 SPSS18.0 统计软件进行数据分析。计量资料采用平均数 $\pm$ 标准差($\bar{x} \pm s$)表示,资料符合正态分布,两组组间比较采用 t 检验,多组组间比较采用 F 检验;以 $P<0.05$ 为具有统计学意义。

2 结果

2.1 各组血清 PCT 水平的动态变化

各组第 1、3、6 天血清 PCT 水平整体比较有统计学差异($P<0.05$),第 9 天血清 PCT 水平整体比较无统计学差异($P>0.05$)。SIRS 组、脓毒症组患儿的第 1、3、6 天血清 PCT 水平显著高于对照组,且随时间延长 PCT 水平逐渐降低,至第 9 天降至正常水平($P<0.05$),而脓毒症组与 SIRS 组间血清 PCT 水平无统计学差异($P>0.05$)。见表 1。

表 1 三组血清 PCT 水平比较(ng/L , $\bar{x} \pm s$)

Table 1 Comparison of serum PCT level in three groups(ng/L , $\bar{x} \pm s$)

Groups	1 d	3 d	6 d	9 d
Control group(n=30)	0.30 $\pm$ 0.12	0.28 $\pm$ 0.15	0.34 $\pm$ 0.19	0.27 $\pm$ 0.17
SIRS group(n=30)	1.92 $\pm$ 1.38*	1.53 $\pm$ 1.26* ^a	1.29 $\pm$ 1.32* ^{ab}	0.31 $\pm$ 0.58 ^{abc}
Sepsis group(n=30)	2.01 $\pm$ 1.24*	1.49 $\pm$ 1.34* ^a	1.21 $\pm$ 1.15* ^{ab}	0.34 $\pm$ 0.54 ^{abc}
F	4.213	6.721	7.156	1.483
P	0.034	0.017	0.015	0.139

Note: Compared with before treatment, * $P<0.05$; Compared with 1 d, ^a $P<0.05$; Compared with 3 d, ^b $P<0.05$; Compared with 6 d, ^c $P<0.05$.

2.2 各组血清 EM 水平的动态变化

脓毒症组第 1、3、6 天血清 EM 水平均高于 SIRS 组($P<0.05$),第 9 天两组血清 EM 水平比较差异无统计学意义($P>0.05$)。脓毒症组 EM 水平随时间延长而降低,至第 9 天降至 SIRS 组的水平($P<0.05$)。而对照组血清 EM 并未达到 ELISA 试剂盒的最低检测限,未检出。见表 2。

2.3 脓毒症组、SIRS 组细胞免疫功能比较

与 SIRS 组相比,脓毒症组的 CD3⁺T 细胞数量增多($P<0.$

05),两组 CD4⁺、CD8⁺T 细胞数量、CD4⁺/CD8⁺ 比例比较无统计学差异($P>0.05$),见表 3。

3 讨论

脓毒症的治疗对临床医生来说是一个挑战,为了提高脓毒症患者的治疗效果,2012 年对国际严重脓毒症和脓毒症休克指南进行了重新修订^[11,12]。尽管有现代复苏策略和抗感染治疗,脓毒症的发病率和病死率仍然很高,成功治疗的关键仍然是早

表 2 三组血清 EM 水平比较(nmol/L, $\bar{x} \pm s$)

Table 2 Comparison of serum EM level in three groups(nmol/L, $\bar{x} \pm s$)

Groups	1 d	3 d	6 d	9 d
Control group(n=30)	ND	ND	ND	ND
SIRS group(n=30)	1.65± 1.24	1.32± 1.19	1.21± 1.01	0.81± 0.58
Sepsis group(n=30)	9.81± 2.39*	7.48± 2.53* ^a	3.19± 1.95* ^{ab}	0.93± 0.61 ^{abc}
t	16.599	12.068	4.938	0.781
P	0.000	0.000	0.000	0.438

Note: Compared with before treatment, *P<0.05; Compared with 1 d, ^aP<0.05; Compared with 3 d, ^bP<0.05; Compared with 6 d, ^cP<0.05.

表 3 脓毒症组、SIRS 组细胞免疫功能比较($\bar{x} \pm s$)

Table 3 Comparison of cellular immune function in sepsis group and SIRS group($\bar{x} \pm s$)

Groups	CD3 ⁺ (%)	CD4 ⁺ (%)	CD8 ⁺ (%)	CD4 ⁺ /CD8 ⁺
SIRS group(n=30)	59.27± 7.2	43.64± 4.56	19.39± 3.59	2.25± 0.29
Sepsis group(n=30)	63.56± 7.53	39.40± 2.37	22.16± 5.39	1.77± 0.11
t	3.466	1.807	1.697	1.528
P	0.001	0.076	0.095	0.132

期诊断脓毒症^[13-15]。生物标志物的检测可对临床决策和预测脓毒症相关的结果提供很大的帮助,目前大量研究正在试图寻找特异性强、敏感度高的早期诊断脓毒症生物标志物^[16-18]。目前临床上应用的诊断脓毒症和判断预后的生物标志物是 C 反应蛋白(C reactive protein, CRP)和 PCT^[19,20]。CRP 是一种判断炎症反应的标志物,CRP 在肝脏中合成,由白细胞介素-6(IL-6)对组织损伤、炎症、感染刺激做出反应所触发,正常人体内含量较少,据有关研究显示,当人体受到组织损伤或感染以后,血清 CRP 水平将显著上升,甚至可以上升千倍^[21]。CRP 已在临床上广泛使用多年,但它的特异性不高,血清 CRP 水平在鉴别细菌、病毒、衣原体、支原体感染以及非感染炎症疾病中缺乏特异性^[22]。

PCT 前体由肝、肾、肌肉以及脂肪细胞等实质细胞释放,机体暴露于毒素后,血清 PCT 在 2 h-4 h 开始上升,大约 14 h 达高峰。PCT 作为临床上广泛使用的脓毒症生物标志物,特异性比 CRP 更好,但有研究发现 PCT 在非感染性的条件下也会升高,如外伤、外科手术、胰腺炎和肾功能损害等^[23,24]。一些研究表明^[25,26],心脏骤停和复苏后血浆 PCT 会升高,但和细菌感染没有关联。有研究对 3000 多例拟诊为脓毒症患者进行研究,发现 PCT 对所有脓毒症患者的可靠诊断缺乏足够的敏感度,阴性时排除脓毒症的特异性也不高,PCT 对脓毒症的预测价值有限,寻找脓毒症的新的生物标记物已成为目前研究的热点^[27-29]。

EM 存在于哺乳动物细胞和组织中,并且是公认的免疫细胞活性的调节因子,在维持机体对应激反应的内环境平衡起重要作用。本研究结果发现,第 1、3、6 天脓毒症患儿和 SIRS 患儿的血清 PCT 水平均高于健康儿童,并且 SIRS 组和脓毒症组患儿的 PCT 水平随时间延长而降低(P<0.05),而脓毒症组和 SIRS 组之间的血清 PCT 水平差异无统计学意义(P>0.05),表明 PCT 作为脓毒症的生物标志物之一,特异性不强,并不能区分脓毒症和 SIRS。ELISA 法检测各组血清 EM 水平,结果显示,脓毒症组的血清 EM 水平,在第 1、3、6 天,显著高于 SIRS

组(P<0.05),其特异性较 PCT 好,能够区分脓毒症和 SIRS,且脓毒症组 EM 水平随时间延长而降低,可以作为脓毒症早期诊断的生物标志物之一。本研究也发现,脓毒症组血清 EM 水平显著增加的同时,外周血 T 淋巴细胞亚群(CD3⁺)百分数比 SIRS 显著增加(P<0.05),与 Horvath RJ 等^[30]的研究结果相符,EM 与其它因素协同能增加免疫活性。

综上所述,EM 在脓毒症患儿血清中水平显著增加,CD3⁺T 细胞数量增多,EM 有可能成为脓毒症的新的生物标记物。

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